Remarks

Applicants acknowledge and appreciate the September 24, 2007 interview between the Examiner and the undersigned.

Enablement

In the instant Office Action, claims 1-4, 7-11 and 14 are pending and have been rejected under 35 USC § 112 based on the contention that the specification does not fully enable their scope. In imposing this rejection, it is asserted that the relevant art area is unpredictable, and that U.S. Patent No. 5,001,051 discloses that treatment of cancer is extremely dose dependent. From this the Examiner has argued that the occurrence of side effects due to chemotherapeutic toxicity is highly unpredictable outside of a very narrow dose range.

In response, Applicants first submit that, irrespective of whether the general occurrence of some side effects associated with chemotherapeutic toxicity is unpredictable, the present claims are drawn specifically to reducing alopecia caused by cylcophosphamide administration. Therefore, the presently pending claims have a significantly refined focus relative to the panoply of side effects that may or may not occur across various dose ranges for the broad spectrum of distinct chemotherapeutic agents known in the art.

Applicants further submit that a skilled artisan would know that alopecia is a predictable side effect of cyclophosphamide administration.. In support of this, Applicants courteously direct the Examiner's attention to the enclosed declaration under 37 CRF § 1.132 from Dr. Youcef M. Rustum, who is an inventor on the present application. As is evident from Dr. Rustum's declaration (Item 3) and the references referred to therein and submitted herewith, alopecia is a predictable side effect that has long been known to regularly occur with cyclophosphamide treatment. For example, as declared by Dr. Rustum, the reference of Cline (Cancer Nursing (June, 1984) p221 - 228) establishes that alopecia is known to be a common side effect of cylcophasphamide administration, and that it occurs in the vast majority (75-90%) of patients receiving cyclophasphamide via intravenous administration. Furthermore, as Dr. Rustum has declared, the reference of Seipp (Cancer Principles & Practice of Oncology, (1977) Vol. 2, 5th Edition, Devitta, Jr., Hellman & Rosenberg Editors, p. 2757-2758) discloses that

alopecia is common after two cycles of cyclophosphamide administered at recognized chemotherapeutic doses. Thus, notwithstanding the disclosure of U.S. Patent No. 5,001,051, Applicants submit that one skilled in the art at the time the present application was filed would know that alopecia is a predictable side effect of cyclophosphamide administration.

Applicants note it is asserted in the Office Action that, based on the reference of Sieja (Pharmazie, 55: 958-959, 2000) one would have expected selenium to have no effect on alopecia caused by cyclophosphamide administration.

In response, Applicants point out that the reference of Seija et al. describes a clinical trial in which patients were administered a ***combination*** of cisplatin and cyclophosphamide (see page 1, left column, lines 9-10). Details of this trial are provided under the heading "Experimental" on page 959, left column, lines 12-15, where the therapy is described as "multi-drug chemotherapy" and indicated to comprise cisplatin and cyclophosphamide. Therefore, it is clear that the effects of selenium on reducing alopecia caused by administration of cyclophosphamide alone were not evaluated in this reference, and that Seija et al. is not relevant to establishing pertinent expectations of one skilled in the art at the time the present application was filed. (Nevertheless, Applicants concur that the presently claimed invention would be not be expected by one skilled in the art at the time the present application was filed.)

The Examiner asserts that the specification provides no guidance for reducing hair loss outside of the specific regimes actually tested. In imposing this rejection, it is notable that the Examiner has recognized that determining proper dosage, administrative routes and schedules generally requires no more than routine experimentation on the skilled artisan's part. However, in this particular case, the Examiner has deemed the degree of unpredictability involved in the invention to be extreme, and has therefore contends that this cases warrants "the unusual finding of lack of enablement for general, unspecified dosages and administrative routes/schedules." (See page 5 of the Office Action).

In response, Applicants appreciate the Examiner's recognition that imposing a lack of enablement rejection for the present claims is unusual. However, Applicants submit that Dr.

Rustum's declaration and the aforementioned references cited therein establish on the record that alopecia is in fact a predictable side effect of cyclophosphamide administration, and therefore the present application does not subsist in an extremely unpredictable art area. In view of this, Applicants submit that determining dosage regimes for the presently claimed selenium containing agents for use in reducing alopecia caused by cyclophosphamide administration would not require undue experimentation, given the benefit of the present disclosure. In support of this, the Examiner is directed to Dr. Rustum's declaration (Item 4) and the enclosed reference of Fakih et al. (Cancer Chemother Pharmacol. (2007 Nov 8) [Epub ahead of print]) on which Dr. Rustum is an author. As Dr. Rustum declares, this reference discloses a phase I study for determining recommended doses of selenomethionine that consistently result in protective plasma selenium concentrations. Applicants submit that it is self-evident that conducting clinical studies are well within the purview of those skilled in the art. Following the discovery of the protective effect of selenium on cyclophosphamide-induced alopecia by the present inventors, it is clear that optimizing the dosage, administration and scheduling of treatment with seleno-L-methionine and methylselenocysteine can be routinely performed by clinicians using standard procedures. Accordingly, undue experimentation is not required to practice the invention commensurate with the full scope of the instant claims. Removal of the stated rejection is therefore respectfully requested.

<u>Definiteness</u>

All of the claims stand rejected under 35 USC § 112 as being indefinite (page 6 of the Office Action). The Examiner asserts that claim 1 and claim 8 do not specify any specific therapy or type of therapy in which the claimed dosages are effective, and that therefore "therapeutically effect dose" (claim 1) and "therapeutic dose" (claim 8) are indefinite terms. However, the Examiner has conceded that the specification provides guidance of the term "effective" within the context of the treatment of cancer.

In response, Applicants respectfully point out that claim 1 and claim 8 are drawn to a method for reducing alopecia induced by the administration of cylcophosphamide "to an individual in need of treatment". Accordingly, the terms "therapeutically effect dose" (claim 1)

and "therapeutic dose" (claim 8) refer to an individual who is in need of cyclophosphamide treatment, and as set forth herein and in Dr. Rustum's declaration, effective amounts of cyclophosphamide are known and/or can be determined by routine experimentation. Thus, Applicant submit the claims are not indefinite. If the Examiner is of the opinion that there are any remaining issues in allowing the present application, the Examiner is encouraged to contact the undersigned by telephone.

Conclusion

Based on the above amendments, Applicants believe that claims 1-4, 7-11 and 14 are now in a condition for allowance and therefore respectfully request the Examiner to allow these claims.

Applicants herewith request a two-month extension of time to file this response. Any additional fees due may be charged to Deposit Account No. 08-2442.

Respectfully submitted,
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Prevention of chemotherapy-induced alopecia: A review of the literature

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ABSTRACT Alopecia is a common yet distressing side effect of cancer chemotherapy. Many methods to prevent this hair loss have been reported in the literature. This article focuses on the theoretical basis for using either the scalp tourniquet, scalp hypothermia, or a combination of both to prevent chemotherapy-induced alopecia. Additionally, it provides a comprehensive review of research studies utilizing these methods. Tables summarizing and comparing the various research studies are presented. Limitations of these studies are addressed with recommendations for future research and practice.

Chemotherapy-induced alopecia continues to be a psychologically distressing side effect for the cancer patient undergoing treatment. Depending upon the degree of importance placed on the hair by the patient, alopecia can cause negative changes in body image, decreased social activity, and altered interpersonal relationships.[3,27] Because nurses today are actively involved in both administering chemotherapy and preventing deleterious side effects, methods available to prevent chemotherapy-induced alopecia are of interest. This review of literature will focus on the following areas: 1) the conceptual framework underlying the use of a scalp tourniquet or scalp hypothermia to prevent chemotherapy-induced alopecia; 2) research published in this area; and 3) implications for nursing research and practice.

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Conceptual Framework

Cancer chemotherapy exerts its effect on both malignant and normal cells which have a high mitotic activity. Rapidly dividing normal cells frequently affected by chemotherapy include those in the bone marrow, epithelial lining of the mouth and gastrointestinal tract, and hair follicles. At any given time, 90% of human hair follicles are in the anagen or actively dividing phase; therefore, it is this large proportion of hair follicles that is susceptible to chemotherapy's deleterious effects.[6]

Hair loss from chemotherapy occurs either by total atrophy and loss of the hair root bulb or, more frequently, by partial atrophy of the bulb causing constriction of the hair shaft.[6] The hair shaft then breaks off easily with any trauma such as washing or combing. Alopecia induced by chemotherapy is reversible. Regeneration of hair growth occurs within 1–2 months after discontinuation of therapy.[4]

The degree of hair loss from chemotherapy is both drug and dose-dependent. Doxorubicin and cyclophosphamide are two of the more notorious epilators. Clinical trials document a consistent 85–100% hair loss by patients receiving doxorubicin.[2,23] Furthermore, severe alopecia occurs in 75–90% of patients receiving intravenous cyclophosphamide.[5,20] Other agents known to cause varying degrees of alopecia include: vincristine, actinomycin D, bleomycin, daunomycin, methotrexate, 5-fluorouracil, hydroxyurea, mitomycin C, and VP-16-213.[28]

Methods utilized to prevent chemotherapy-induced alopecia include the scalp tourniquet, scalp hypothermia, or a combination of both. Theoretically, the scalp tourni-

quet will temporarily constrict circulation to the superficial scalp vessels thereby decreasing the amount of chemotherapy able to perfuse the hair follicles. The rationale for the use of scalp hypothermia is similar. Scalp cooling will cause vasoconstriction of the scalp vessels, thus minimizing drug contact with the scalp and hair follicles. This method has the additional theoretical benefit of reducing the temperature-dependent cellular uptake of drugs such as doxorubicin.[14] Also, scalp cooling may decrease the metabolic rate of hair follicles making them less susceptible to the toxic effects of chemotherapy drugs. Ideally, combining both methods will potentiate the benefits of each.

In theory, these methods need to be utilized only during peak plasma drug levels. For example, doxorubicin has a relatively short initial half-life of approximately 30 minutes.[11,29] Presumably, the scalp would need to be protected only during this time. Conversely, cyclophosphamide has a plasma half-life of over 6 hours after being metabolized by the liver.[4,5] This extended half-life would necessitate lengthy use of a scalp tourniquet or scalp hypothermia in order to sufficiently protect the scalp.

When using the scalp tourniquet or scalp hypothermia, the type of neoplastic disease process must be considered. These methods are not recommended in patients with either hematologic neoplasms which have a high incidence of scalp metastases (i.e., leukemia, lymphoma) or solid tumors with known scalp metastases.[28,29] Since these techniques may prevent delivery of cytotoxic agents to both normal hair follicles and harboring tumor cells in the scalp, the procedural risk to each individual patient must be weighed carefully.

In summary, the rationale for utilizing scalp hypothermia and/or scalp tourniquet to prevent chemotherapy-induced alopecia is based primarily on normal hair growth patterns, theorized local effects to the scalp, and drug pharmacokinetics. In addition, the type of neoplastic disease process must also be considered when instituting these techniques.

Review of Literature

Early studies of scalp tourniquet use were primarily anecdotal. Hennessey[13] used an inflatable scalp tourniquet on breast cancer patients receiving cyclophosphamide intravenously. Just prior to chemotherapy injection, the scalp tourniquet was inflated to 10 mm Hg above the patients systolic blood pressure. It then remained inflated for 5 minutes. Hennessey reported a continuance of "some hair loss," but no further instances of complete alopecia since using the tourniquet.

O'Brien et al.[22] utilized the same tourniquet method and timing as Hennessey in children with cancer receiving vincristine. They report "obvious" alopecia developed in only three out of 20 children. Unfortunately, neither Hennessey nor O'Brien et al. utilized a control group to compare hair loss. In addition, terms such as "obvious" or "complete" alopecia were not operationally defined.

Using a minimum scalp tourniquet pressure of 240 mm Hg just prior to and for at least 7 minutes after drug injection, Lyons[19] reported no alopecia in 20 patients treated with a 5-day course of cyclophosphamide, 5-fluorouracil, vincristine, and methotrexate. No rationale was given for the extremely high tourniquet pressure used in this uncontrolled study nor were drug dosages specified.

One of the first comparative studies utilizing a control group was done by Pesce et al.[24] Their nonrandomized trial included 73 patients receiving similar doses of doxorubicin, cyclophosphamide, and vincristine or VM-26. Tourniquet pressure was maintained at 30–50 mm Hg above systolic pressure for 5 minutes prior to and 20 minutes after chemotherapy. In the tourniquet group, 67.5% (n = 25) had "no or minimal hair loss" while 32.5% (n = 12) experienced alopecia. In the control group, 29% (n = 9) had "no or minimal hair loss" while 71% (n = 22) experienced alopecia. Operational definitions of alopecia and "no or minimal hair loss" were not given. Additionally, these results are clouded by the unexplained exclusion of five patients from the final data.

Soukop et al.[25] compared scalp tourniquet use in patients receiving similar doses of doxorubicin, cyclophosphamide, and vincristine. In the randomly assigned experimental group (n = 14), the tourniquet was inflated to 10 mm Hg over systolic pressure immediately before and for 30 minutes after chemotherapy administration. No tourniquet was used in the control group (n = 19). Final results indicated that alopecia was universal in both groups. However, the mean time to alopecia was delayed in the tourniquet group (10.3 weeks) as compared with the control group (4.2 weeks).

Focusing on doxorubicin-induced alopecia, Lovejoy[17] inflated a scalp tourniquet to 50 mm Hg above systolic pressure prior to, during and for 15 minutes after drug administration. Evaluation of hair loss took place after cumulative doses of 180–315 mg/m^2 were reached. Three independent judges viewed patient photographs and graded hair loss according to 5% intervals. Patients in the experimental group experienced 17 ± 14% hair loss (range = 1–26% while the control group exhibited 69 ± 32% hair loss (range = 33–95%). The significance of these results is limited by the extremely small sample size of only six patients.

Holmes[15] compared the effectiveness of a penrose scalp tourniquet in a nonrandomized trial of 29 patients receiving doxorubicin and/or cyclophosphamide. The rubber tourniquet was applied by the patients in the experimental group 5 minutes before, during, and after chemotherapy for a total of 15 minutes. Patients were

instructed to save any hair lost on a daily basis. Investigators counted hair loss samples to determine average daily hair loss. The control group exhibited a significantly larger daily hair loss than the experimental group. However, despite comparable cyclophosphamide doses between groups, a greater number of patients in the control group ($n = 11$) received doxorubicin at larger doses (mean = 74.09 mg; median = 70 mg) than the experimental group ($n = 8$; mean = 51.75 mg; median = 40 mg). This weakens the significance of these results. Not surprisingly, average daily hair loss was significantly greater at cumulative doses larger than 200 mg of doxorubicin and 3,500 mg of cyclophosphamide. This supports the direct correlation between hair loss and drug dosage.

Finally, Maxwell[21] found no statistically significant difference in tourniquet versus no tourniquet use in nine male patients receiving cyclophosphamide, methotrexate, vincristine, and actinomycin. In the experimental group, the tourniquet was inflated to 10 mm Hg above systolic pressure just prior to treatment. Patients were instructed how to control the tourniquet pressure themselves. As a result, tourniquet pressure and timing during and after chemotherapy administration were variable. This inconsistency in methodology weakens the significance of the study results.

Concurrent research of scalp hypothermia has focused solely on preventing doxorubicin-induced alopecia. Luce et al.[18] devised a plastic helmet attached to a room air conditioner to cool patients' scalps to 18–28°C 5 minutes prior to and 10–20 minutes after doxorubicin injection. The 12 patients who underwent scalp cooling lost approximately 30% of their hair as opposed to 80% hair loss in the control group ($n = 16$). Drug dosages were not specified and evaluation of hair loss took place after only one treatment.

Edelstyn et al.[10] molded frozen cryogel bags to the scalp for 10 minutes before and 30 minutes after administration of doxorubicin, vincristine, and 5-fluorouracil. Of the 77 randomized patients, 81% (30/37) of the control group had "severe to total alopecia" as compared with only 50% (20/40) of the scalp cooling group experiencing the same degree of hair loss. Methods utilized to define the degree of alopecia were not specified. Mention was made to the researchers' difficulty in keeping the cryogel bags in position which they felt resulted in inadequate cooling.

Using crushed ice in plastic bags, Dean et al.[7] cooled patients' scalps to 23–24°C 5 minutes prior to and 30 minutes after intravenous doxorubicin administration. Evaluation of hair loss was done prior to each treatment by both the patient and nurse examiner utilizing a graded rating scale and subject photographs. Overall, 20 out of 33 patients had "good protection" (25–50% hair loss) against alopecia throughout all cycles of treatment. Dean compares this success rate with a 95% incidence of total alopecia in an historical control group of similar patients. Significantly, the degree of protection against hair loss was inversely proportional to the dose administered, i.e., those receiving greater than 50 mg of doxorubicin experienced decreasing protection with each course of treatment. Similar results were reported in a replication study by Dean et al.[8] utilizing a commercial product, Kold Kap® instead of crushed ice.

Timothy et al.[26] anecdotally report success in using ice packs applied to the scalp 20 minutes before and 20 minutes after doxorubicin injection. They cite two instances where patients maintained their scalp hair using hypothermia despite almost complete epilation of pubic and axillary hair.

More recently, Anderson et al.[1] devised a cooling cap of frozen polyethylene gel packs molded together and applied to the scalp 15 minutes before and at least 30 minutes after chemotherapy. This method is unique because it includes first wetting the patient's hair and covering it with a wet crepe bandage "to reduce the amount of trapped air under the cap and thus improve conduction." Treatment drugs included doxorubicin and vincristine or vindesine. Evaluation of hair loss was done prior to each course and at the end of treatment by the research nurse using a graded rating scale. No control group was used. Of the 28 patients who underwent scalp cooling, 12 had "no substantial hair loss" and 10 experienced only "minor hair loss." Interestingly, of the nine patients with liver function abnormalities, six experienced severe to total alopecia. The authors suggest scalp hypothermia may not be effective in patients with decreased liver metabolism due to prolonged plasma concentration of doxorubicin.

Guy et al.[12] utilized a plastic nylon cap with liquid coolant flowing from a thermocirculator. Scalp cooling to 25°C was initiated 15 minutes prior to treatment with doxorubicin, vincristine, cyclophosphamide, and methotrexate. Cooling continued for 30 minutes after drug injection. Evaluation of hair loss was done by patients and investigators using subject photographs. Of the 12 patients studied, eight had "no or very slight hair loss," while four had "slight to moderate hair loss." Again, no control group was used.

Finally, Dugan[9] studied the scalp hypothermia effectiveness of the commercial product, Chemocap.® However, the tourniquet included with the Chemocap® was not utilized. The nonrandomized study groups included patients with oat cell carcinoma of the lung receiving similar doses of doxorubicin, cyclophosphamide, and vincristine. No significant difference in hair loss was noted between the Chemocap® versus no-Chemocap® groups. All patients had severe hair loss after 6 weeks. An important influencing factor may be the addition of total brain irradiation during the fifth week. Certainly, the effect of radiation-induced alopecia must be considered in these findings.

TABLE I
Summary of Scalp Tourniquet Studies

Source	Hennessey (1966)	O'Brian et al. (1970)	Lyons (1974)	Pasce et al. (1978)	Soukop et al. (1978)	Lovejoy (1979)	Holmes (1979)	Maxwell (1980)
Randomization	—	—	—	—	+	+	—	—
Control group	—	—	—	+	+	+	+	+
Inflatable tourniquet pressure	10 mm Hg above systolic BP	10 m Hg above systolic BP	240–300 mm Hg	30–50 mm Hg above systolic BP	10 mm Hg above systolic BP	50 mm Hg above systolic BP	? Pressure—Penrose tourniquet	10 mm Hg above systolic BP
Tourniquet timing (minutes) before/after drug injection	JP/5	JP/5	JP/7	5/20	JP/30	JP/15	5/5	JP/variable
Chemotherapy agents and doses (mg)	CYC 100 mg I.V. × 2 days 500 mg I.V. × 5 days	VCR 1.5 mg/m² I.V.	CYC VCR MTX 5FU I.V. Doses: N/S	DOX 30–50 mg I.V. CYC 300–600 mg I.V. × 3 days VCR1–2 mg I.V. VM-26 50 mg I.V.	DOX 50 mg/m² I.V. CYC 500 mg/m² I.V. VCR 1 mg/m² I.V.	DOX 60–100 mg I.V.	DOX 30–90 mg I.V. CYC 480–1200 mg I.V.	CYC 40 mg/kg I.V. MTX 0.6 mg/kg I.V. VCR 2 mg. I.V. ACT 2 mg I.V.
No. of courses	N/S	5–7	1	≥4	1–6	≥2	≥4	≥4
No. of subjects	N/S	30	20	73[a] E = 37 C = 31	33 E = 14 C = 19	6 E = 3 C = 3	29 E = 15 C = 14	9 E = 5 C = 4
Assessment parameters for measuring alopecia	N/S	N/S	N/S	N/S	When patient began wearing a wig	Subject photos, independent judges, graded scale (5% intervals)	Counted hair loss samples	Subject photos, independent judges, graded scale 0–100%
Results	No further complete alopecia since using scalp tourniquet	Obvious alopecia 3 out of 30 patients	No alopecia among 20 patients	No or minimal hair loss E = 25 (67.5%) C = 9 (29%) Alopecia E = 12 (32.5%) C = 22 (71%)	Mean time to alopecia E = 10.3 wks. C = 4.2 wks.	Hair loss E = 17 ± 14% (1–26%) C = 69 ± 32% (33–95%)	Average daily hair loss E = 311 hairs C = 1792 hairs	No significant difference in hair loss between groups

[a] Five patients unaccounted for.
Notes: N/S = not specified; JP = just prior to drug injection; E = experimental group; C = control group; ACT = actinomycin; CYC = cyclophosphamide; DOX = doxorubicin; 5FU = 5 fluorouracil; MTX = methotrexate; VCR = vincristine; VM-26 = teniposide.

Only one study has combined the use of both scalp hypothermia and scalp tourniquet. Kennedy et al.[16] also studied the commercial product, Chemocap,® but utilized both the cooling cap and the scalp tourniquet. After being applied to the scalp for 10 minutes, the special frozen cap is temporarily removed and a noninflatable scalp tourniquet applied. The combination treatment continues for an additional 10 minutes prior to chemotherapy and for 30 minutes after drug injection. Treatment drugs included doxorubicin and/or cyclophosphamide. This randomized controlled trial revealed no significant difference in hair loss between control ($n = 9$) versus experimental ($n = 10$) groups. Because all five subjects with liver metastases (experimental = 4; control = 1) had alopecia after one or two treatments, the authors also suggest these methods may be ineffective in patients with liver function abnormalities.

Discussion

This review of literature on preventing chemotherapy-induced alopecia has revealed some promising initial studies. However, prior to utilization of this research in oncology nursing practice, some shared study limitations must be addressed. First, there are widely varying tourniquet pressures used in the scalp tourniquet studies (Table I). This may be due to the lack of physiological studies which specifically define optimal scalp tourniquet pressure. Nevertheless, such a wide range of tourniquet pressures in these studies makes it difficult to compare study results accurately.

As summarized in Table II, the scalp hypothermia studies utilize a variety of scalp cooling methods ranging from crushed ice to liquid coolant. Whether one cooling method is superior cannot be ascertained from these initial studies. Again, the lack of consistency in cooling methods and resulting temperatures limits direct comparison between studies.

Common to both scalp tourniquet and scalp hypothermia research are variations in timing schedules used. As illustrated in Table I, prechemotherapy timing of the scalp tourniquet is fairly consistent—usually just prior to drug injection. However, the amount of time the tourniquet remains inflated after drug injection is much more variable, ranging from 5 to 20 minutes. In the scalp hypothermia studies, prechemotherapy cooling studies range from 5 to 20 minutes, while postchemotherapy cooling approximates 30 minutes (Table II). Optimally, the timing schedule of either method should be based on drug pharmacokinetics as discussed previously. However, with the current trend toward combination chemotherapy, multiple drug half-lives often need to be considered. Not only do these differences in timing schedules limit comparison of these studies, they also point out the need for more in-depth investigation into drug pharmacokinetics when planning proper timing of these methods.

It is also important to note the different types and doses of chemotherapy agents used in these studies. Most of the more recent research has focused on preventing doxorubicin-induced alopecia which, theoretically, may be the most preventable due to its unique short initial half-life and temperature-dependent cellular uptake. However, two important factors need to be considered here. First, in addition to doxorubicin, most of the patients in these studies are concurrently receiving numerous other chemotherapy drugs which also cause varying degrees of alopecia. Additionally, many of these trials study different doses of doxorubicin (Tables I, II, and III). Since several studies have supported the occurrence of greater hair loss at higher cumulative doses of doxorubicin and cyclophosphamide,[8,15] these drug factors tend to cloud study results if not controlled properly. Both the type and dose of chemotherapy must be considered carefully not only when designing future research studies, but also when weighing the significance of study results.

Another methodological weakness of these studies is the lack of randomized, controlled trials. Only five out of 16 studies utilize a randomized control group, while an additional four studies utilize a control group, but without random assignment. As noted with Holmes'[15] scalp tourniquet trial, lack of randomization can result in a disproportionate number of patients in one group receiving larger drug doses. Certainly, further trials of these methods would be strengthened by the inclusion of randomization and control groups within the study design.

The inherent difficulty in attempting to measure or quantify hair loss is a universal problem in these studies. As summarized in Tables I, II, and III, various assessment methods have been utilized to measure alopecia. These range from counting individual hairs to grading hair loss from subject photographs. Assessment of pretreatment hair condition (i.e., amount, thickness, balding patterns, etc.) is addressed inconsistently. Most of the assessment parameters, if even specified, vary from study to study with rare attention to their reliability and validity.

After assessment of hair loss, terms such as "minimal" or "significant" alopecia or graded scales have been used to further quantify to degree of alopecia experienced. These terms and graded scales also vary from study to study and are inconsistently operationally defined. Once again, it is difficult to compare the findings as a whole due to inconsistent assessment and measurement criteria.

This review of literature has addressed numerous problematic areas evident in the published research on prevention of chemotherapy-induced alopecia. These include study variations with regard to tourniquet pressure, cooling methods, time schedules, type and dose of chemotherapy, randomization and control groups, and hair loss assessment and measurement parameters. These

[a] Five patients unaccounted for.
Notes: N/S = not specified; JP = just prior to drug injection; E = experimental group; C = control group; ACT = actinomycin; CYC = cyclophosphamide; DOX = doxorubicin; 5FU = 5 fluorouracil; MTX = methotrexate; VCR = vincristine; VM-26 = teniposide.

TABLE II

Summary of Scalp Hypothermia Studies

Source	Luce et al. (1973)	Edelstyn et al. (1977)	Dean et al. (1979)	Dean et al. (1981)	Anderson et al. (1981)	Guy et al. (1982)	Dugan (1983)
Randomization	—	+	—	—	—	—	—
Control group	+	+	—[a]	—[a]	—	—	+
Scalp cooling method	Regional chilled air	Frozen cryogel bags	Crushed ice	Kold Kap®	Frozen polyethylene gel packs	Liquid coolant via thermocirculator	Chemocap® (without tourniquet)
Scalp cooling time (minutes) before/after drug injection	5/10–20	10/30	5/30	5/30	15/≥ 30	15/30	20/30
Chemotherapy agents and doses (mg)	DOX (Dose N/S)	DOX 50 mg I.V. VCR 2 mg I.V. 5FU 500 mg I.V. MTX 5 mg po × 4 days CHLOR 10 mg po × 4 days	DOX 30 mg/m² I.V. CYC 150 mg/m² po × 4 days	DOX 30 mg/M² I.V. CYC 150 mg/m² po × 4 days	DOX 40–60 mg I.V. VCR 2 mg I.V. VND 5 mg I.V.	DOX 50 mg/m² I.V. VCR 1–4 mg/m² I.V. MTX 40 mg/m² I.V.	DOX 40 mg/m² I.V. CYC 1000 mg/m² I.V. VCR 1 mg/m² I.V. (total brain irradiation, week 5)
No. of courses	1	1	≥5	≥5	≤8	≥4	2
No. of subjects	28 E = 12 C = 16	77 E = 40 C = 37	33	25	28	12	11 E = 6 C = 5
Assessment parameters for measuring alopecia	Maximum % hair loss was estimated	N/S	Subject photos graded scale	Subject photos graded scale	Subject photos graded scale	Subject photos graded scale	Subject photos
Results	Average hair loss E = 30% C = 80%	Hair loss E / C Total 13 / 23 Severe 7 / 7 Slight 17 / 7 No 3 / 0	Protection from hair loss Excellent (0–25% loss) to good 25–50% loss) poor (75–100% loss)	Ice / Kold Kap® 23 / 18 10 / 7	Hair loss No significant Minor Severe or total	N / Hair loss 12 / No or slight 10 6 / Slight to moderate	N 6 4
							No significant difference in hair loss between groups

[a] Used historical control group.

Notes: N/S = not specified; E = experimental group; C = control group; CHLOR = chlorambucil; CYC = cyclophosphamide; DOX = doxorubicin; 5FU = 5 fluorouracil; MTX = methotrexate; VCR = vincristine; VND = vindesine.

limitations by careful controlled netics; 3) r and 4) ope Addi chemothe to determi patients v needs to b condition hair condi and break would not influence trition, su such as a studies. (merous p with whic these me Clea with trer aptation t direct uti must be

Notes: E = phosphamide

Assessment for measuri alopecia Results

No. of subjec

Chemotherap agents and doses (mg)

drug injectic before/after

Timing (minut

Method

control grou

Randomizatio

Source

TABLE III

Summary of Combination Study

Source	Kennedy et al. (1983)
Randomization	+
control group	+
Method	Chemocap®
Timing (minutes) before/after drug injection	Tourniquet 10/30 cooling cap 20/30
Chemotherapy agents and doses (mg)	DOX 20–125 mg I.V. CYC 300–900 mg I.V.
No. of courses	2–6
No. of subjects	19 E = 10 C = 9
Assessment parameters for measuring alopecia	Subject photos graded scale
Results	Occurrence of alopecia (After 2 treatments) E = 80% $n = 8$ C = 77% $n = 7$ No significant difference

Notes: E = experimental group; C = control group; CYC = cyclophosphamide; DOX = doxorubicin.

limitations could be addressed in future research studies by careful consideration of the following: 1) randomized, controlled study design; 2) drug dosage and pharmacokinetics; 3) reliability and validity of assessment instruments; and 4) operational definitions of the degree of hair loss.

Additionally, the influence of liver dysfunction on chemotherapy-induced alopecia should be investigated to determine if these treatment modalities are feasible in patients with liver metastases. Another variable which needs to be addressed is the influence of pretreatment hair condition on hair loss from chemotherapy, i.e., will poor hair condition potentiate alopecia due to increased damage and breakage. Perhaps those patients with damaged hair would not be optimal candidates for these techniques. The influence on hair loss by such such variables as malnutrition, surgical anesthesia, and concurrent medications such as anticoagulants must also be addressed in future studies. Only by further research, resolving these numerous problematic areas, will definitive data be available with which to predict optimal individual patient benefit from these methods.

Clearly, prevention of alopecia is an exciting concept with tremendous implications for improving patient adaptation to chemotherapy. However, at the present time, direct utilization of these methods in the clinical setting must be done carefully and cautiously with grave consideration of the limitations of these studies. Without more definitive research on the ability of the scalp tourniquet and/or scalp hypothermia to prevent chemotherapy-induced alopecia, it is impossible—except anecdotally—to weigh patient benefit against the risks of patient discomfort, patient safety, and consumer cost. As patient advocates, researchers, and consumers of research, nurses have a responsibility to use prudent judgment when incorporating these techniques into clinical practice. □

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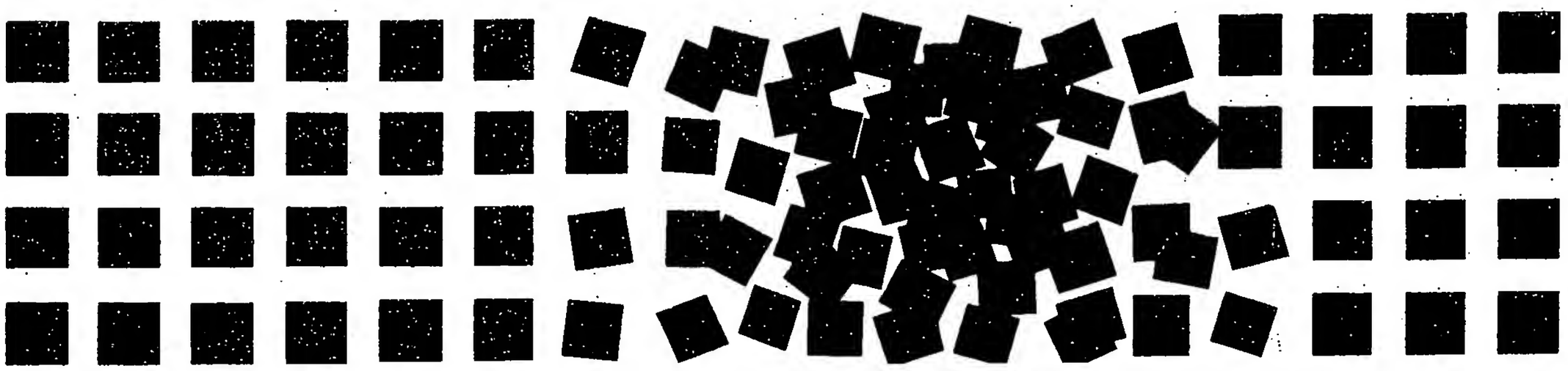

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SECTION **6**

CLAUDIA A. SEIPP

Hair Loss

Alopecia is a psychologically distressing, yet common side effect of many chemotherapeutic agents and radiation therapy. As patients embark on new therapies, hair loss can induce a negative body image, alter interpersonal relationships, and arouse enough anxiety to cause some patients to reject potentially curative treatment. In one study, 88 percent of women who received perioperative chemotherapy for early breast cancer considered alopecia to be the most burdensome aspect of therapy.[1]

Frank discussion of the problem by clinicians and oncology nurses with recognition of the patient's stress is helpful in preparing the patient to confront this loss.[2] Although current methods for the prevention of total scalp hair loss or the use of wigs after hair loss are not entirely satisfactory for all patients, caregivers can offer psychological support and some practical suggestions. Often the presence of a spouse, family member, or friend during this discussion with the patient is helpful in placing the problem in perspective.

The hair loss caused by scalp irradiation is unpredictable. Epilation can begin at doses of 500 cGy and generally progresses with spotty areas of baldness as the course of treatment continues. The prospects for hair regrowth diminish with increasing doses.[3] Radiation ports on extremities have been noted to be hair-free 10 years after radiation therapy and may never have hair regrowth. In lower-dose ranges regrowth begins 8 to 9 weeks after cessation of therapy. Patients should be cautioned that the new hair may be different in character from the pretreatment hair.[4]

The extent of body hair loss by patients in any chemotherapeutic program is both drug- and dose-dependent and is related to the frequency of cycle repetition Often it is caused by more than one drug being used concurrently (Table 53.6-1). Long-term therapy may result in loss of pubic, axillary, and facial hair as well as scalp hair. It should be emphasized to patients that alopecia from chemotherapy is reversible, with hair regeneration beginning 1 to 2 months after therapy is discontinued. Alteration in color and texture of hair may occur: hair may be a lighter or darker shade and is often curlier as it regrows.[6] Hair loss may begin 1 to 2 weeks after a single chemotherapeutic dose and reaches maximum loss within 2 months in most drug sequences. Doxorubicin and cyclophosphamide are common cytologic agents known to cause epilation after two cycles at doses of doxorubicin above 50 mg/m^2 and cyclophosphamide above 500 mg/m^2. Although agents differ in the degree to which they cause hair loss, alopecia may be expected with other single-agent antibiotics, alkylators, nitrosoureas, and especially their combinations.[7]

HEAD COVERING

Most patients choose to cover their heads during periods of hair loss. Nurses and clinicians can suggest wigs or head covering with stylish scarves, turbans, or hats. Wigs should be selected before hair loss begins so that the patient is prepared when alopecia occurs and so that hair color and style can be matched. Hairpieces are tax-deductible medical expenses and are covered by some medical insurance policies. Several small private businesses have been developed by former patients who distribute or sell head coverings of various designs. An American Cancer Society (ACS) rehabilition program called "Look Good, Feel Better" has been developed in partnership with the National Cosmetology Association and the Cosmetic, Toiletry and Fragrance Association Foundation specifically to assist women to compensate for hair loss and skin changes during cancer treatment.[8] Volunteer beauticians and cosmetologists help women look better and feel more comfortable with changes in their appearance such as dry, discolored, or blotching skin, discolored nails, and alopecia. Information about group programs and their location is available through an ACS hotline 1–800–395–LOOK. The support program is active in all 50 states and Puerto Rico with a group designed for teenagers anticipated in the future.

PREVENTION OF ALOPECIA

Since 1966, interventions have been proposed to prevent scalp hair loss from chemotherapy.[9] The rationale for these procedures is to prevent drug circulation to the hair follicles by causing temporary vasoconstriction and decreasing tissue metabolism at the time of peak plasma drug level, with either an occlusive scalp tourniquet or localized hypothermia. The pharmacokinetic profiles of the drugs to be used should be understood before either of these methods is considered. Scalp cooling systems must maintain temperatures below 22°C to have any effect. Occlusion of the superficial scalp veins must begin before the drugs are given and, to be effective, must be extended beyond the time of the peak plasma drug levels.[10–12]

Various types of scalp icing devices have been manufactured by several different American companies. Although the Food and Drug Administration had initially approved the marketing of cooling caps intended to cause localized scalp hypothermia, early in 1990 the FDA reviewed these applications and became concerned that the safety and efficacy of these devices had not been substantiated by adequate clinical data.[5,13–17] Regulatory action was initiated to address the following concerns:

- The potential for scalp metastasis posed by the use of these devices
- The potential for reducing drug circulation to other anatomic sites beyond the scalp such as the skull and possibly the brain
- The effectiveness of preventing hair loss and how specific cytologic doses and other variables affected the results achieved. Therefore, the FDA halted the commericial distribution of these devices, and 5 years after their withdrawal, no company has come forward with supporting clinical evidence of reasonable safety and effectiveness, according to Frances Moreland Curtis of the Division of General and Restorative Devices, FDA (written communication, August 1995).

TABLE 53.6-1. Single Agents With Potential to Induce Reversible Alopecia*

Amsacrine	5-Fluorouracil
Bleomycin	Hydroxyurea
Cyclophosphamide	Ifosfamide
Dactinomycin	Methotrexate
Daunorubicin	Mitoxantrone
Doxorubicin	Mitomycin
Epirubicin	Melphalan
Etoposide	Paclitaxel
Vincristine	Vinblastine
5-Fluorodeoxyuridine (FUDR)	

* The degree or onset of alopecia is inversely proportional to dose, schedule of sequences, rate of delivery, route of delivery, and various combinations of agents used concurrently.[5]

Although there are indications that continuous flow systems with thermostatically controlled cooling caps are still used in Europe, they seem to be limited to regimens containing a single anthracycline alopecia-inducing agent.[17] Limitations of safety and inconclusive and conflicting reports of the results of the usefulness of scalp hypothermia should be factors discussed with patients seeking information about these devices and hair preservation techniques.

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SECTION 7

Gonadal Dysfunction

For young adults who have cancer the success of treatment with regimens that are toxic to gonadal function has made infertility an important problem. When the cancer is controlled, quality of life then becomes a major issue. To many of these young persons, quality of life includes their ability to have a normal child. This is especially so because many of the life-threatening or debilitating effects of cytotoxic therapies can now be mitigated.

Both neoplastic disease and its treatment interfere with normal sexual and reproductive function (Table 53.7-1). Testicular and ovarian cancer directly involve the gonad, and prostate, endometrial, and cervical cancer directly involve the reproductive tract. Surgical treatment for any of these diseases results by necessity in the loss of these important reproductive organs. Retroperitoneal lymph node dissection (RPLND) for testicular and colon cancer, prostatectomy, and surgery involving the bladder neck may result in loss of the ability to ejaculate. Primary and metastatic tumors in the hypothalamus and pituitary can directly affect gonadotropin secretion, resulting in secondary hypogonadism. Both chemotherapy and radiation can cause a variety of toxic effects on the male and female gonads. Cytotoxic therapies delivered to women during pregnancy can have teratogenic effects on the fetus, the discussion of which is beyond the scope of this chapter.[1,2] If fertility is maintained or recovers, there remains the concern about the heritability of cancer and at least a theoretical risk of mutagenic alterations to germ cells caused by cytotoxic therapies. In addition, social and behavioral responses to cancer and its therapy affect sexuality.

The reproductive consequences of cancer therapy affect many people. In the United States, 9100 male patients ages 15 to 35 are diagnosed each year with Hodgkin's disease, lymphoma, bone and soft tissue sarcomas, testicular cancer, and leukemia.[3] Of these, about 1400 are treated with high doses of procarbazine, cyclophosphamide, radiation, or cisplatin sufficient to induce prolonged azoospermia. Similarly 60,000 females between 15 and 50 are diagnosed with breast cancer (mostly), Hodgkin's disease, lymphoma, and leukemia; at least 80% of these patients are treated with radiation or alkylating-agent–based cytotoxic therapies. These treatments cannot only cause sterility, but also premature menopause and the associated estrogen deficiency. In addition, 7500 children under 15 are diagnosed each year with cancer, including leukemia, nervous system tumors, lymphomas, and other solid tumors.[4] Survival is approaching 80%, and since 85% of these children receive chemotherapy or gonadal or pituitary irradiation, reproductive dysfunction is a significant concern.

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ORIGINAL ARTICLE

A Phase I and pharmacokinetic study of selenomethionine in combination with a fixed dose of irinotecan in solid tumors

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Abstract

Purpose We conducted a phase I study to determine the recommended dose of selenomethionine (SLM) in combination with irinotecan that consistently results in a protective plasma selenium (Se) concentrations > 15 μM after 1 week of SLM loading.

Experimental Design A 3-3 standard escalation design was followed. SLM was given orally twice daily (BID) for one week (loading) followed by continuous once daily (QD) dosing (maintenance). Seven dose levels of selenomethionine were investigated. Irinotecan was given intravenously at a fixed standard weekly dose, starting on the first day of maintenance SLM.

Results Thirty-one patients were treated on study. Dose limiting diarrhea complicated by sepsis was noted in one of six patients at each of the dose-levels 1 and 7. Dose-levels ≥ 5 (4,800 mcg/dose loading maintenance) resulted in day 8 Se concentrations >15 μM while dose-level 7 (7,200 mcg/dose loading and maintenance) resulted in day 8 Se concentrations > 20 μM. No significant variations in SN-38 or biliary index were noted between weeks 1 and 4 of treatment. Despite achieving target Se concentrations, gastrointestinal and bone marrow toxicities were common and irinotecan dose modification was prevalent. Objective responses were seen in two patients and nine patients had disease control for 6 months or longer.

Conclusions Selenomethionine can be escalated safely to 7,200 mcg BID × 1 week followed by 7,200 mcg QD in combination with a standard dose of irinotecan. No major protection against irinotecan toxicity was established; however, interesting clinical benefits were noted-supporting the investigation of this combination in future efficacy trials.

Keywords Selenomethionine · Irinotecan · Pharmacokinetics · Phase I · Colon cancer

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Introduction

Selenium is an essential trace element with a worldwide average nutritional intake of 50–350 μg/day. Dietary selenium deficiency has been associated with an increased risk of carcinogenesis and of increased mortality [1, 2]. Multiple other epidemiological studies have suggested that higher Se blood levels are protective against the development of various solid tumors [3–12]. Clark et al. [13] investigated the use of 200 μg/day of Se (as selenized yeast) as a chemoprevention agent for non-melanoma skin cancer.

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Selenium supplementation resulted in a significant reduction in total cancer mortality and total cancer incidence including lung, colorectal, and prostate cancers [13, 14]. Furthermore, a retrospective analysis of baseline Se levels in patients with non-Hodgkin's lymphoma showed a positive correlation between plasma Se levels and chemotherapy dose-delivery and outcome [15]. In a multivariate analysis, Se was the most important factor affecting survival with a hazard ratio of 0.76 for every 0.2 μM increase in concentration [15].

Given the favorable epidemiological data and the decreased chemotherapy-related toxicity in patients with normal or elevated serum selenium concentrations, our group has investigated the utility of high dose selenium supplementation with chemotherapy in pre-clinical models. We have shown that the organic selenium compounds SLM and methylselenocysteine (MSC) decrease irinotecan-induced toxicity in nude mice while at the same time increasing irinotecan antitumor activity [16]. The optimal protective effects of SLM against chemotherapy toxicity were noted when SLM was started 1 week prior to chemotherapy [16]. Furthermore, this protection against normal tissue toxicity was found to be dose dependent for both MSC and SLM with the threshold protective Se concentration with SLM being 15 μM [17, 18].

Based on these encouraging preclinical data, we initially conducted a phase I clinical trial of a fixed dose of SML at 2,200 μg/day (μg of elemental Se) in combination with escalating doses of weekly irinotecan [19]. Contrary to our expectations, we were not able to escalate irinotecan beyond its established recommended dose of 125 mg/m^2/week [19]. However, interesting clinical responses were noted on this study, particularly in a previously irinotecan-resistant patient who achieved the highest plasma concentration of Se [19]. Pharmacokinetic analysis on our initial phase I study confirmed that plasma concentrations of Se after 1 week of 2,200 μg/day of SLM loading (on the first dose of irinotecan administration) were suboptimal (< 10 μM), suggesting that higher doses of SLM are needed to test adequately for normal tissue toxicity protection [19].

We have thus designed and conducted a sequel phase I clinical trial to determine the optimal dose of SLM that results in Se concentrations exceeding the 15 μM threshold for protection against toxicity. Based on our prior pharmacokinetic modeling and the estimated 1 month lag for steady state Se concentration with daily administration of SLM [19], we elected to investigate a 1 week BID SLM loading schedule followed by a lower QD SLM maintenance dosing with the goal of achieving our target 15 μM Se concentration by the end of the loading phase. We maintained irinotecan dosing at a fixed recommended dose of 125 mg/m^2/week to be started on the eighth day of SLM, i.e. after completion of the loading SLM phase. Our two main objectives were to determine the minimum and the maximum safe dose of SLM that results is Se plasma concentrations exceeding 15 μM. Our rationale behind escalating SLM beyond doses achieving the target protective threshold were based on the selenium dose-dependent antitumor synergy detected between irinotecan and organic selenium compounds [17, 18].

Materials and methods

This phase I, open-label, dose-escalation study of SLM in combination with a fixed dose of irinotecan was conducted at Roswell Park Cancer Institute (Buffalo, NY). The primary objective of the study was to determine the lowest and highest safe doses of SLM among seven dose-levels that result in Se plasma concentrations exceeding 15 μM on days 8 and 29 of SLM when combined with a fixed dose of weekly intravenous irinotecan. Secondary objectives included the evaluation of pharmacokinetics of SLM and irinotecan, the description of treatment-related toxicities, and the description of any observed clinical responses.

Patient criteria

Patients with a histologically or cytologically confirmed solid tumor that was metastatic or unresectable and for which standard curative or palliative measures did not exist or for whom single agent irinotecan constituted a reasonable treatment option were eligible for the trial. The last chemotherapeutic or radiation treatment was at least 4 weeks (6 weeks for nitrosureas or mitomycin C) prior to trial enrollment. Other criteria included age ≥ 18 years of age, ECOG performance status ≤ 1, estimated life expectancy > 12 weeks, no central nervous system involvement, adequate bone marrow function (neutrophils ≥ 1,500/μL, hemoglobin ≥ 8.0 g/dull, platelets≥100,000/μL), adequate hepatic function [serum bilirubin ≤ upper limit of normal range (ULN), serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2.5 × ULN], and adequate renal function (creatinine ≤ 1.5 ULN or creatinine clearance ≥ 60 mL/min). The study excluded patients unable to receive oral medications, patients with brain metastases, patients with a history of Gilbert's syndrome, and patients with active inflammatory bowel disease or chronic diarrhea. HIV positive patients were not eligible because of possible pharmacokinetic interaction with anti-retroviral drugs. Patients with≥G2 neuropathy (NCI CTC 3.0) were excluded because of concerns about possible exacerbation of neurotoxicity with SLM. Patients with reproductive potential had to agree to use adequate contraception prior to study entry and for the duration of study participation. The study and consent form

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were approved by the institutional scientific and review committee (SRC) and the institutional review board (IRB) prior to its activation. All patients provided signed informed consent before study entry. The study was conducted in accordance with the good clinical practice guidelines as issued by the international conference on harmonization and the declaration of Helsinki.

Study design and treatment plan

Dose escalation

Three patients were entered at each dose level. In the absence of dose limiting toxicity (DLT), the next dose level was explored. If DLT was seen in one patient, three further patients were added at that dose level and, if no additional DLT was seen, escalation to the next dose level occurred. If at least two patients had DLT at a given dose level, accrual to that dose level was stopped; this was the maximally administered dose. Further patients were then added, as required, to the previous dose level (and if necessary to lower dose levels) to establish the highest dose at which < 2/6 patients had DLT. This was the maximum tolerated dose (MTD). No dose escalation was allowed beyond dose level 7 as the number of pills to be administered per day beyond that level was thought to be limiting. In the event that escalation to dose level 7 was feasible and no DLT were noted in the first 3 patients at that dose level, dose level 7 was to be expanded to 6 patients to determine if this was the MTD among the 7 levels investigated. No intra-patient escalation was allowed.

Treatment schedule

Selenomethionine was given PO twice daily (loading phase) starting 1 week prior to the first dose of irinotecan and subsequently once daily (maintenance phase). SLM was administered in the form of 800 or 400 mcg capsules (Sabinsa Inc.). Seven dose levels of SLM were to be investigated (Table 1). Irinotecan was administered intravenously (i.v.) at a fixed dose of 125 mg/m^2 in 500 cc of normal saline (NS) over 90 minutes once weekly × 4 every 6 weeks (one cycle) [20]. Patients were medicated with dexamethasone 10 mg (i.v.) and palonosetron 0.25 mg (i.v.) prior to irinotecan.

Table 1 Dose levels of selenomethionine

Phase I escalation schema			
Dose level	SLM loading (D-7–D-1)[a] (mcg PO BID)	SLM maintenance (D1 and on) (mcg PO QD)	Irinotecan (mg/m^2) Q week (start on D1)
1	3,200	2,800	125
2	3,200	3,200	125
3	4,000	3,200	125
4	4,000	4,000	125
5	4,800	4,800	125
6	5,600	5,600	125
7	7,200	7,200	125

[a] Day 1 follows day-1 (no day 0)

Dose limiting toxicities

A dose limiting toxicity was any of the following attributable to study treatment on cycle 1: any non-hematological grade (G) 3 or 4 toxicity, with the exception of G3 diarrhea lasting less than 48 h; any G4 thrombocytopenia or any G3 thrombocytopenia lasting more than 6 days; any G4 neutropenia lasting more than 6 days or any G4 neutropenia associated with fever; any dose-delay secondary to toxicity that lasts 2 or more weeks or results in giving less than 3 of the 4 scheduled weekly irinotecan treatments on the first cycle. G3 hypomagnesemia, G3 hypophosphatemia, G3 hypokalemia, and sodium levels of 128–130 mmol/l were not considered DLT unless they were persistent for more than 48 h despite medical intervention or in case they resulted in hospitalization.

Dose modifications

Dose modifications for irinotecan were required for G2 and higher toxicities (Table 2). Treatment was interrupted for any G3 or higher toxicity; missed treatments were not made up. A cycle was not to be started unless the absolute neutrophil count (ANC) recovered to ≥ 1,500/ml and the platelets to ≥ 75,000/ml and non-hematological treatment related toxicities improved to ≤ G1. Patients were instructed to take loperamide 4 mg PO at the onset of diarrhea and 2 mg every 2 h until diarrhea resolved. No growth factors were allowed on the study with the exception of recombinant erythropoietin.

No dose modification was allowed for SLM; however, the total daily dose of SLM could have been divided into 2–3 doses/day in case of dyspepsia.

Clinical evaluation and follow-up

A complete medical history, physical examination, pregnancy test for women with reproductive potential, complete blood count (CBC), and comprehensive chemistry profile (electrolytes, BUN, creatinine, magnesium, lactate dehydrogenase, ALT, AST, bilirubin) were obtained within a week prior to treatment initiation. Baseline CT scans were obtained within 4 weeks prior to initiation of treatment. CBC and comprehensive chemistry were repeated on a

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Table 2 Irinotecan dose modifications for hematological and non-hematological toxicities

	During same cycle	During next cycle
No Toxicity	Maintain dose level	Maintain dose level
Grade 1	Maintain dose level	Maintain dose level
Grade 2[a]	Reduce by 25 mg/m^2 for dose level 1. Reduce to prior dose level for dose level 2 and above	Maintain dose level for dose level 1. Reduce to prior dose level for dose level 2 and above
Grade 3	Omit until Grade 2 or less and then decrease by 25 mg/m^2 for dose level 1. Reduce to prior dose level for dose level 2 and above	Decrease by 25 mg/m^2 for dose level 1. Reduce to prior dose level for dose level 2 and above
Grade 4	Omit until Grade 2 or less and then decrease by 50 mg/m^2 for dose level 1. Decrease dose by 40 % for dose level 2 or above	Decrease by 50 mg/m^2 for dose level 1. Decrease dose by 40% for dose level 2 or above

No dose-modification indicated for correctable electrolyte disturbances, hyperglycemia, vomiting that has not been treated with the maximum antiemetic therapy, alopecia, or toxicities that are not related to study drugs (irinotecan and SLM) but do not interfere with drug metabolism or clearance (example grade 3 pain secondary to bony metastases)

[a] Grade 2 hematological toxicities did not require dose modification on the next cycle

weekly basis on the first cycle (including the two-week break) and prior to planned irinotecan treatments on subsequent cycles. Medical history, physical examination, and toxicity assessment as per NCI CTC 3.0 were performed weekly on the first cycle and on weeks 1 and 3 of subsequent cycles. CT scans were repeated every 2 cycles (12 weeks) to assess response. Responses were categorized according to the RECIST criteria [21].

SLM treatment compliance was evaluated via a combination of a study specific patient diary and a monthly pill count.

Pharmacokinetics: sample collection, preparation, and analysis

Sample collection

Trough samples of blood for Se levels were collected in trace element free heparinized tubes on days 1, 2, 8, and 28 prior to the administration of SLM. Multiple samples of blood for phamacokinetic determinations of CPT-11, SN-38 and SN-38G were collected in separate heparinized tubes on week 1 of irinotecan administration and again on week 4 to evaluate potential effects of Se on CPT-11 pharmacokinetics and metabolism.

Selenium measurements

Selenium in plasma was measured by graphite furnace atomic absorption spectrophotometry using PE ZL4100 or PE analyst as has been described previously [19]. Quality assurance was maintained by running quality control samples assayed every time the patient samples are run as described earlier [19].

Measurement of irinotecan (CPT-11), SN-38 and SN-38G

CPT-11, SN-38 and SN-38G were measured using reverse phase HPLC with fluorescence detection as described on our prior phase I clinical trial with identical sample preparation and HPLC conditions [19].

Pharmacokinetic data analysis:

PK analysis of the concentration-time data for CPT-11, SN-38 and SN-38G was carried out using non-compartmental methods using WinNonlin version 5.0 (Pharsight Corporation, Lexington, KY). Specific concepts behind the calculations of some of the derived parameters have been described before [19]. Summary statistics and comparisons wee made using SAS statistical software (PROC Mixed, SAS version 8.02, Cary, NC).

Results

Demographics

Between October 2004 and June 2006, 31 patients (27 evaluable) were entered on study. Four patients were not evaluable for treatment induced toxicity and are detailed below. One patient withdrew from study on her third week of treatment because of symptoms of progressive disease. One patient with a large ventral hernia developed a bowel incarceration early in her treatment that was deemed unrelated to study drugs and was taken off study. One patient was taken off study because of non-compliance with SLM treatment in the loading part of the study. The last non-evaluable patient was taken off study on day 1 of

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Table 3 Patient characteristics

Patient characteristics (n = 27 evaluable)	
Gender (male/female)	20/7
Age (median/range)	57/21–74 years
ECOG (0/1)	16/11
Primary tumor	
Colorectal	22
Small lung cancer	2
Non-small cell lung cancer	1
Sarcoma	1
Urachal	1
Prior chemotherapy	27
Prior irinotecan chemotherapy	12
Prior radiation therapy	10

irinotecan as she was found to have pre-existing hallucinations that were related to narcotic treatment. These four patients were excluded from the toxicity analysis and efficacy analysis, as they were withdrawn from study prior to completing the first cycle of treatment. None of these four patients had evidence of treatment related toxicity at the time of their withdrawal from study. The characteristics of the 27 evaluable patients are listed in Table 3.

Treatment administration

Thirty-one patients received treatment on study, of whom, 27 are evaluable. All seven-dose levels of SLM were investigated. The median number of cycles administered was 2 (range 1–8), with a total of 90 cycles administered on study. Six patients received 6 or more cycles. All patients received all intended SLM treatment without any scheduling modification. However, dose modification of irinotecan was common. Nineteen patients required irinotecan dose interruption or reduction during the first cycle due to treatment toxicity-as mandated per study protocol.

Toxicity

Twenty-seven patients were evaluated for treatment-related toxicity. Only ≥ G2 toxicity data attributed to study treatment are reported. Treatment-related G2–G4 toxicities are summarized in Tables 4 and 5.

Hematological toxicity

Neutropenia was the predominant hematological toxicity. Cycle 1 G3 neutropenia was noted in one patient at dose level 1, one patient at dose level 3, and 3 patients at dose level 7. No G2 or above thrombocytopenia was noted on treatment. Hematological toxicities are detailed in Table 4.

Non-hematological toxicity

The most common ≥ G2 non-hematological adverse event was diarrhea. Six patients experienced G2 diarrhea and 5 experienced G3 diarrhea on cycle 1. G3 diarrhea occurred in one patient at DL1, 2 patients at DL5, and 2 patients at DL7. Grade 3 diarrhea lasted > 24 h in only 2 patients (DLT defining), one on DL1 and the other on DL7. Other common non-hematological toxicities included nausea and vomiting, fatigue, and abdominal cramps. Non-hematological toxicities for cycle 1 and for all cycles are detailed in Table 5.

Selenomethionine toxicity

Selenomethionine was well tolerated in all patients. The only toxicity attributed to SLM was mild garlic-like odor (breath and urine) and was limited to G1 in about 50% of the patients. This was seen more commonly during the induction SLM week and tended to ameliorate or disappear with prolonged treatment. No skin or nail toxicities secondary to SLM were documented.

Dose limiting toxicities, maximum tolerated dose, and recommended dose

Two patients experienced a dose limiting toxicity as defined by the study protocol. One patient with extensive peritoneal carcinomatosis experienced G3 abdominal pain, G3 nausea and vomiting, G3 diarrhea, G3 neutropenia, and G3 infection on his 3 week of treatment on cycle 1. Although his symptoms were partly attributed to disease progression

Table 4 Hematological toxicities (≥ grade 2)

Toxicity	DL1 (6pts) (G2/G3/G4)	DL2 (3pts) (G2/G3/G4)	DL3 (3pts) (G2/G3/G4)	DL4 (G2/G3/G4)	DL5 (G2/G3/G4)	DL6 (G2/G3/G4)	DL7 (G2/G3/G4)
Neutropenia cycle 1	1/1/0	2/0/0	0/1/0	0/0/0	0/0/0	0/0/0	1/3/0
Neutropenia all cycles	1/1/0	1/1/0	0/1/0	0/0/0	0/0/0	0/0/0	1/3/0
Anemia cycle 1	1/0/0	0/0/0	1/0/0	1/0/0	0/0/0	0/0/0	0/0/0
Anemia all cycles	1/0/0	0/0/0	1/0/0	1/0/0	0/0/0	0/0/0	0/0/0

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Table 5 Non-hematological toxicities

Toxicity	DL1[a] (G2/G3/G4) $n = 6$	DL2 (G2/G3/G4) $n = 3$	DL3 (G2/G3/G4) $n = 3$	DL4 (G2/G3/G4) $n = 3$	DL5[b] (G2/G3/G4) $n = 3$	DL6 (G2/G3/G4) $n = 3$	DL7[bc] (G2/G3/G4) $n = 6$
Diarrhea cycle 1	2/1/0	1/0/0	1/0/0	1/0/0	0/2/0	1/0/0	0/2/0
Diarrhea all cycles	4/1/0	2/0/0	2/0/0	1/0/0	0/2/0	1/0/0	0/3/0
N/V Cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	2/0/0	3/0/0
N/V all cycles	0/1/0	0/0/0	0/0/0	0/0/0	1/0/0	2/0/0	3/1/0
Fatigue cycle 1	0/1/0	1/0/0	1/0/0	0/0/0	1/0/0	1/0/0	2/0/0
Fatigue all cycles	0/1/0	1/0/0	1/0/0	1/0/0	1/0/0	1/0/0	3/0/0
Anorexia cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	1/0/0	3/0/0
Anorexia all cycles	0/1/0	0/0/0	0/0/0	1/0/0	0/0/0	1/0/0	4/0/0
Abdominal cramps cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	1/0/0	1/0/0	2/0/0
Abdominal cramps all cycles	0/1/0	0/0/0	0/0/0	0/0/0	1/0/0	1/0/0	2/0/0
Constipation cycle 1	0/0/0	0/0/0	0/0/0	0/0/0	1/0/0	0/0/0	2/0/0
Constipation all cycles	0/0/0	1/0/0	1/0/0	0/0/0	1/0/0	0/0/0	2/0/0
Infection cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/1/0
Infection all cycles	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/1/0
Hyponatremia cycle 1	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0	0/1/0
Hyponatremia all cycles	0/0/0	0/0/0	0/1/0	0/0/0	0/0/0	0/0/0	0/1/0
Dyspepsia cycle 1	0/1/0	0/0/0	0/0/0	0/0/0	0/0/0	1/0/0	0/0/0
Dyspepsia all cycles	0/1/0	0/0/0	0/0/0	0/0/0	1/0/0	1/0/0	0/0/0
Hypotension cycle 1	0/0/0	0/0/0	1/0/0	0/0/0	1/0/0	0/0/0	0/0/0
Hypotension all cycles	0/0/0	0/0/0	1/0/0	0/0/0	1/0/0	0/0/0	0/0/0

[a] One patient on dose level 1 experienced a dose limiting toxicity consisting of diarrhea, nausea and vomiting, fatigue, sepsis (complicating grade 3 neutropenia), dyspepsia, abdominal cramps/pain, and anorexia. All G3 toxicities on dose level 1 involved the same patient

[b] Two G3 diarrhea on dose level 5 and one G3 diarrhea on dose level 7 lasted less than 24 h and thus did not constitute a DLT

[c] One patient of dose level 6 experienced a dose limiting toxicity consisting of diarrhea, sepsis (complicating G3 neutropenia), and hyponatremia

with partial small bowel obstruction, treatment related toxicity could not be ruled out as a contributing factor. DL1 was expanded to 6 patients without any further DLT. Further escalation of SLM did not result in any further DLT until DL7. At DL7, none of the first 3 patients experienced a DLT. Since DL7 was the highest dose level to be investigated, this cohort was expanded to 6 patients to determine if it fits the maximum tolerated dose (MTD) definition. The sixth patient on DL7 experienced a DLT. This patient, similar to the patient with DLT on DL1, suffered from extensive peritoneal carcinomatosis. On her second week of treatment, she experienced symptoms of partial small bowel obstruction with G2 N/V and DLT defining G3 infection, neutropenia, diarrhea, and hyponatremia.

The non-tolerable dose of SLM was not defined on this study. The MTD of SLM among the seven dose levels investigated was defined as DL7, consisting of SLM at 7,200 mcg PO BID × 1 week followed by 7,200 mcg PO QD. Given the tolerability of DL7 and the achievement of the target Se concentrations at that dose level (see PK section), DL7 was also declared the recommended dose for future studies.

Antitumor activity

Twenty-five patients were evaluable for radiographic response. The two patients with DLT did not have confirmed radiographic progression but suffered from symptoms of peritoneal carcinomatosis progression with the development of partial small bowel obstruction. Two patients had a partial response (PR). Both patients had a diagnosis of colorectal cancer and both had previously progressed on 5-FU, leucovorin, oxaliplatin, and bevacizumab but had no prior irinotecan exposure. The responses lasted for 7 and 10 months from initiation of study treatment. Twelve patients had stable disease (SD), 6 of which were confirmed with subsequent CT scans as per RECIST criteria. The 6 confirmed SD lasted between 7 and 12 months. Five of the 6 patients had a diagnosis of metastatic colorectal cancer and one patient had non-small cell lung cancer. Four patients with confirmed SD had prior irinotecan exposure including one case with prior irinotecan refractory disease.

Pharmacokinetics

Day 8 Se levels are available for 31 patients and Day 28 levels for 21 patients.

Pharmacokinetic data for CPT-11, SN-38 and SN-38G are available for 26 patients on Week 1 of irinotecan administration (7 days after the loading dose administration of SLM) and 11 patients on week 4 of irinotecan administration (28 days after the maintenance dose of SLM).

Selenium levels in plasma

Levels of Se in plasma of patients after a loading dose of SLM for 7 days and after 3 weeks of maintenance dosing are presented by dose level of SLM administered in Table 6. As evident from the data, while there is a significant inter-patient variability in selenium levels achieved by day 8, all patients had Se levels greater than 15 μM by dose level 5 (4,800 μg BID). All patients at dose level 7 had greater than 20 μM Se concentrations in plasma by day 8. The mean ± SD of Se levels at dose levels 5 ($n = 3$), 6 ($n = 4$) and 7 ($n = 6$) on day 8 were 20.8 ± 3.0, 16.94 ± 2.5 and 30.7 ± 10.6 μM, respectively. There was no significant further accumulation of Se on the maintenance dose administered for further 3 weeks until dose level 7, as evident from the Se levels in plasma measured on day 28. At dose level 7, for the 4 patients where data were available on day 8 and day 28, the mean ± SD of Se in plasma on day 8 and 28 were 26.7 ± 7.6 and 34.09 ± 9.0 μM respectively.

Serum Se levels was measured every 6 weeks in patients who remained on study for more than one cycle. Selenium levels reached a plateau in these patients at levels similar or slightly higher than the day 28 levels.

Irinotecan pharmacokinetics

With a goal to determine whether chronic administration of Se affects the PK and metabolism of irinotecan, the pharmacokinetics of CPT-11 and its major metabolites SN-38 and SN-38G have been evaluated after the first dose of irinotecan (week 1), and again after the fourth dose (week 4) (Table 7). It is apparent from the data that all the derived PK parameters for CPT-11 and the metabolites are unchanged between week 1 and week 4 when the patients are on the daily dose of SLM for 3 weeks. A trend towards decline in SN-38 AUC is suggested from the data and calculated biliary index, but is not statistically significant ($p = 0.31$). While the biliary index is a measure of conversion of SN-38–SN-38G relative to its synthesis from CPT-11, the data do not suggest any increase in SN-38G AUC.

Day 8 plasma selenium concentration and toxicity

The protective effects of plasma Se on irinotecan induced gastrointestinal and bone marrow toxicity in the evaluable population was explored by stratifying patients into 3 groups according to their Day 8 (day of first irinotecan dose) Se concentration. Since diarrhea and neutropenia are the most prevalent irinotecan related toxicities, only these two toxicities were captured for this exploratory analysis. Eight patients had a suboptimal Se concentration of < 15 μM on Day 8 of SLM; 2/8 had G3 and 5/8 had ≥ G2 diarrhea or neutropenia. Eleven patients had Day

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Table 6 Se levels on day 8 and day 29 in patience receiving selenomethionine

Dose level (loading mcg BID/ maintenance mcg QD)	Pt no	Day 8 selenium level		Day 28 selenium level	
		ng/ml	μM	ng/ml	μM
DL1 (3,200/2,800)	1	785	9.94	866	10.96
DL1 (3,200/2,800)	2	1,647	20.85	1,598	20.23
DL1 (3,200/2,800)	3	1,199	15.18	No sample[a]	–
DL1 (3,200/2,800)	4	1,210	15.32	No sample[a]	–
DL1 (3,200/2,800)	5	900	11.39	1,237	15.66
DL1 (3,200/2,800)	6	1,225	15.51	1,216	15.39
DL2 (3,200/3,200)	7	633	8.01	721	9.13
DL2 (3,200/3,200)	8	1,110	14.05	No sample[a]	–
DL2 (3,200/3,200)	9	1,041	13.18	1,227	15.53
DL2 (3,200/3,200)	10	1,247	15.78	1,195	15.13
DL3 (4,000/3,200)	11	1,028	13.01	No sample[a]	–
DL3 (4,000/3,200)	12	1,298	16.43	No sample[a]	–
DL3 (4,000/3,200)	13	1,250	15.82	1,011	12.80
DL3 (4,000/3,200)	15	1,525	19.30	No sample[a]	–
DL4 (4,000/4,000)	17	1,563	19.78	No sample[a]	–
DL4 (4,000/4,000)	18	919	11.63	1,006	12.73
DL4 (4,000/4,000)	19	1,481	18.75	1,395	17.66
DL5 (4,800/4,800)	20	1,430	18.10	1,413	17.89
DL5 (4,800/4,800)	21	1,608	20.35	1,297	16.42
DL5 (4,800/4,800)	22	1,899	24.04	2,276	28.81
DL6 (5,600/5,600)	23	1,355	17.15	1,481	18.75
DL6 (5,600/5,600)	24	1,605	20.32	1,894	23.97
DL6 (5,600/5,600)	25	1,937	24.52	No sample[a]	–
DL6 (5,600/5,600)	26	1,245	15.76	1,528	19.34
DL7 (7,200/7,200)	27	NC[b]	–	1,662	21.04
DL7 (7,200/7,200)	28	2,089	26.44	2,608	33.01
DL7 (7,200/7,200)	29	2,280	28.86	No sample[a]	–
DL7 (7,200/7,200)	30	2,350	29.75	3,173	40.16
DL7 (7,200/7,200)	31	1,294	16.38	1,719	21.76
DL7 (7,200/7,200)	32	2,700	34.18	3,272	41.42
DL7 (7,200/7,200)	33	3,838	48.58	No sample[a]	–

[a] Day 29 sample was not collected

[b] Non-compliant

8 plasma Se concentrations ranging ≥ 15 and < 20 μM; 3/11 had G3 and 7/11 had ≥ G2 diarrhea or neutropenia. Eight patients had Day 8 plasma Se concentrations ≥ 20 μM; 4/8 had G3 and 5/8 had ≥ G2 diarrhea or neutropenia. Although no formal statistical analysis was performed, higher Se concentrations prior to initiation of irinotecan did not seem predictive of protection against diarrhea or neutropenia.

Discussion

We had previously demonstrated that the administration of daily SLM or MSC, starting one week prior to initiation of weekly irinotecan therapy, reduces irinotecan-induced toxicity and improves antitumor activity in preclinical models [16, 17]. In a previous phase I study we tested the ability of SLM to attenuate irinotecan toxicity by assessing the feasibility of irinotecan escalation beyond the previously recommended MTD of 125 mg/m^2 when combined with a fixed dose of SLM of 2,200 mcg/day in patients with advanced solid tumors [19]. Escalation of irinotecan was not possible on that study secondary to DLT consisting of prolonged G3 diarrhea [19]. However, Se concentrations on the day of initiation of irinotecan were suboptimal in all patients (< 10 μM), significantly less than the optimal concentration of 15 μM and higher [19]. Other findings included interesting clinical benefits in a variety of solid tumors and a reduction in biliary index when comparing week 4–week 1 of irinotecan pharmacokinetics.

We thus conducted this sequel phase I trial to determine the dose of SLM that results in Se concentrations that

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Table 7 Summary of PK parameters for CPT-11, SN-38, and SN-38G

	C_{max} (ng/ml/mg/m^2)	Half-life (h)	V_D (L/m^2)	CL (L/h/m^2)	AUC_{inf} (ng h/ml)	Biliary Index[a]
CPT-11						
Week 1	15.8 (28)	8.6 (22)	91 (37)	11.9 (31)	11,582 (33)	5,450 (122)
Week 4	14.6 (25)	8.3 (13)	86 (21)	13.1 (29)	10,398 (32)	3,123 (47)
SN-38						
Week 1	0.9 (37)	19.5 (64)	2,422 (53)	132.8 (61)	1,256 (60)	–
Week 4	0.9 (64)	16.8 (40)	2,850 (58)	157.6 (57)	1,059 (61)	–
SN-38G						
Week 1	1.8 (40)	14.7 (44)	798 (58)	37.2 (58)	3,483 (49)	–
Week 4	2.1 (34)	14.2 (23)	748 (54)	42.6 (46)	3,446 (38)	–

All values are mean (CV%)

Week 1, $N = 26$; Week 4, $N = 11$

[a] Biliary index = $(AUC_{CPT\text{-}11}\ AUC_{SN\text{-}38})/AUC_{SN\text{-}38G}$

$P = 0.31$ for biliary index between week 1 and 4 (paired t-test)

exceed the threshold of 15 μM and to further evaluate the effects of prolonged SLM administration on irinotecan pharmacokinetics. Simulation analysis of the data from our initial study formed the basis for the design of the current escalation scheme for SLM. It is evident from the data presented that a BID dose of SLM for one week can produce a level of 15 μM concentration in plasma. Much higher levels in the order of ~25 μM are produced at the BID dose of 7,200 mcg, although an occasional individual still shows poor absorption of Se. The accumulation of Se appears to be limited as evident from the day 28 levels except at the highest dose of 7,200 μg SLM.

We evaluated the changes in irinotecan pharmacokinetics by comparing week 4 to week 1 of cycle 1. As evident from the data, no significant changes in PK parameters for either CPT-11 or its metabolites are found from week 1 to week 4. A slight decline in SN-38 AUC is suggested from the data and calculated biliary index, but is not statistically significant ($p = 0.31$). While the biliary index is a measure of conversion of SN-38 to SN-38G relative to its synthesis from CPT-11, the data do not suggest any changes in SN-38G AUC. Since the calculation of biliary index is also tied to the AUC of CPT-11, it is conceivable that changes in biliary index could encompass other metabolic reactions involving CPT-11 itself. We still cannot rule out an effect of SLM on irinotecan PKs based on the current data as both week 1 and 4 PK parameters were obtained in the setting of SLM treatment. It is possible that the difference in biliary index noted in the predecessor study (only 6 patients evaluated) was related to Se interaction with irinotecan that became evident due to the differences in Se concentration between week 1 and 4 [19]. On this current study, there has been no significant difference between Day 8 (week 1 irinotecan) and Day 29 (week 4 irinotecan) Se concentrations and thus, possibly, the lack of evident interaction. The sole way to conclusively study SLM/irinotecan interaction would be to administer irinotecan alone followed by SLM plus irinotecan for a definitive PK interaction study. This was not feasible in our phase I SLM escalation design.

Despite the fact that our study was not designed to investigate the protective effects of SLM on irinotecan-induced normal tissue toxicity, the frequent attenuation of irinotecan dosing on cycle 1 secondary to toxicity suggests lack of major protection. In fact, 19 out of 27 patients required dose reduction in irinotecan in the first cycle of treatment. To explore the possibility that higher Se concentrations may be more protective, we stratified our patient population according to their Se level on the day of their first irinotecan dose. Surprisingly, the rate of G2 and above toxicity did not seem to decrease with higher selenium concentrations. We continued to see significant irinotecan-induced toxicities even at the highest SLM cohort where Se concentrations of 30 μM were seen. This suggests that if SLM has any protective effects against irinotecan toxicity, those protective effects would be minimal. This is in contrast with the pre-clinical data generated by our group where SLM clearly allows the doubling of the maximum tolerated dose of irinotecan in nude mice. The discrepancy between our clinical and pre-clinical findings is poorly understood. It is likely that SLM may have different mechanisms of activity in mice in comparison to humans. Furthermore, despite the correlation between Se concentration and protective effects of SLM in mice, it is unclear that this endpoint is a valid surrogate endpoint of SLM activity in humans. Methylselenol has been previously established as the active metabolite of SLM; however, plasma Se concentrations do not reflect the concentrations of this active metabolite in patients [22]. Due to the instability and volatility of methylselenol, no validated clinical assay has been formulated yet to test this metabolite.

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We have seen a large number of disease stabilizations and two partial responses on this study. Some of the stabilizations were prolonged and occurred in patients with previously documented irinotecan-refractory disease. Although these should be considered anecdotal, these findings would not been inconsistent with the synergy described between irinotecan and organic Se [17].

Future studies should determine which tumors are most likely to benefit for the addition of high dose of SLM to the treatment regimen. Selenomethionine has been shown to significantly alter the expression of 50 genes in the colorectal cancer cell line HCT116 [23]. Others have also shown that p53 status is predictive of the antitumor activity of Se compounds in vitro [24, 25]. Tumor molecular profiling before and after SLM treatment in future therapeutic trials may shed some insight on its mechanisms of activity and biological targets.

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